An All Solid State Potentiometric Sensor for Monohydrogen Phosphate Ions

Pankaj Kumar,¹, ² Dong-Min Kim,¹ Myung Ho Hyun,¹ Mi-Sook Won, b Yoon-Bo Shim* a

¹ Department of Chemistry and Institute of Biophysio Sensor Technology, Pusan National University, Busan 609-735, South Korea
tel: +82 51 510 2244; fax: +82 51 510 2430
² Busan Center, Korea Basic Science Institute, Busan 609-735, South Korea

* Present address: Department of Chemistry, University of Petroleum and Energy Studies, Dehradun, Uttarakhand, India-248007
*e-mail: ybshim@pusan.ac.kr

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Abstract
An all solid state potentiometric sensor using anthracene thiourea derivative as ionophore was developed. It exhibited a near-Nernstian slope of $30.8 \pm 1.0 \text{ mV/decade of activity}$ for $\text{HPO}_4^{2-}$ ions in the concentration range of $1.0 \times 10^{-7} - 1.0 \times 10^{-3} \text{ M}$ at pH 7.4. It displayed excellent selectivity for monohydrogen phosphate over other anions and the selectivity sequence was determined as $\text{HPO}_4^{2-} > \text{SO}_4^{2-} > \text{Cl}^- > \text{NO}_3^- > \text{OAc}^- > \text{I}^- > \text{ClO}_4^-$. The developed sensor was evaluated for the analysis of monohydrogen phosphate ions in a standard reference material (SRM 1548) as well as in the potentiometric titration of phosphate ions with a barium chloride solution.

Keywords: Ionophore, Monohydrogen phosphate, All-solid-state-membrane electrode, Anthracene thiourea derivative, Anion sensor

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1 Introduction
Phosphate concentration monitoring finds wide applications in analytical chemistry, clinical chemistry, pharmacology, and environmental chemistry. Phosphate helps to develop and manage energy, synthesize protein, fat and carbohydrates, contract muscles, and maintain the body’s fluid and electrolyte balance. It is also essential for stimulating hormone production and helping the body utilize the B vitamins. Adenosine triphosphate (ATP) is responsible for all energy production and storage processes within the body [1]. It combines with calcium ions to help the lattice formation for strong bones and teeth. Phospholipids such as lecithin and cephalin are the key components of cell membranes [2]. DNA and RNA also are the long chains of phosphorous containing molecules. In addition, phosphorus is essential in chemical processes involved in the utilization of carbohydrates and fats [3]. It liberates their energy at the rate, demanded by the body. In combination with calcium, it feeds the nerves and also aids the growth of hair, helps counteract fatigue, regular functioning of heart, and for normal kidney functioning. It helps in regulating the acid alkaline balance of the blood which is vital for the maintenance of health. Its deficiency may cause anxiety, bone problems, fatigue, irregular breathing, irritability, numbness, skin sensitivity, stress, teeth weakness, tremors, weakness, worry, and weight changes. Excess of phosphate can cause kidney damage and osteoporosis [4]. Therefore, the precise and simple monitoring of phosphate is highly desired.

There are several techniques available for the determination of phosphate ions, like spectroscopic methods, which involve the formation of molybdenum blue [5]. This complex technique remains unsuitable for the application to automated sensing and feedback control. Another technique of the automated flow analysis [6] requires sophisticated instrumentation and produces toxic laboratory waste. Besides, these methods need large infrastructure back up and are time consuming. Thus, reliable and quick analytical methods are crucial, one of which requirements are ion sensors. A number of phosphate selective potentiometric sensors have been reported in the literature. Several types of active materials have been used for the preparation of phosphate selective electrodes [7–10]. First bivalent phosphate electrode was based on the ion exchange membrane containing amine chloride [11]. Tin(IV) complexes were among the early compounds found suitable as ionophores in the polymeric membrane based phosphate selective electrodes. This category of ionophores included the derivatives of dibenzyltindichloride [12] and multidentate-tin(IV) carrier [13]. Binuclear organotin compounds were also explored later [14]. These electrodes exhibited high sensitivity towards $\text{HPO}_4^{2-}$ ions but a short life time due to the hydrolysis of the ion-carrier in the membrane phase. Also, their selectivity was relatively poor. Uranyl salophanes were also
successfully used as electroactive materials in the PVC matrix membrane electrodes [15–17]. These electrodes suffered with short life time. Cyclic polyamines like 3-decyl-1,5,8-triazacyclodecane-2,4-dione [18,19] have been used as a carrier in polymeric membranes to obtain highly sensitive and selective electrodes for HPO$_4^{2-}$ ions. A ferrocene bearing macroyclic amide based sensor has shown a narrow linear working concentration range of 1.0 x 10$^{-2}$–1.0 x 10$^{-5}$ M [20]. Besides of these, various types of electroactive compounds such as heterocyclic macrocycle; 3-allyl-1,5,8-triazacyclodecane-2,4-dione [21], macrocycles [22], zwitertionics based anionic complex organoborane compound [23], bis-thiourea [24], urea or thiourea-functionalized calix[4]arenes [25], and a macrocyclic dithioamide receptor [26] based electrodes have been reported for the selective quantification of HPO$_4^{2-}$ ions.

Most of the reported potentiometric ion-selective electrodes are of conventional type. These are based on the polymeric membranes and contain an inner reference solution for the development of potential gradient across the membrane. The presence of liquid inside the electrode restricts their applications up to some extent. Also, for robustness and miniaturization, it is desirable to eliminate the liquid phase of inner reference solution from the conventional electrochemical cells to enhance their practical suitability for medicinal, biological sciences and technology, and in vivo experiments [27–29]. All solid state ion-selective polymeric membrane electrodes as well as ISEs containing hydrogels as the inner layers are of particular interest because they are free from the above limitations. To our knowledge, there are only a few all solid state monohydrogen phosphate selective electrode, reported till date [17,30,31]. Therefore, efforts are needed for the development of all solid state sensor for these ions.

Here, we report for the first time a better performing all solid state ISE (ASISE) for monohydrogen phosphate ions with a new ionophore, which exhibits a wide linear range with a long life span. The selectivity coefficients of the electrode were measured for a number of interfering anions such as acetate, chloride, nitrate, sulfate, perchlorate, and iodide. The experimental response curve was obtained by using a 0.05 M phosphate solution with varying pH, and compared with the predicted response curve. The optimized ASISE was successfully used as an indicator electrode in the potentiometric titration of phosphate ions against the standard barium chloride solution as well as yield 0.3 g (70 %) of anthracene thiourea derivative as a yellow solid (Figure 1).

2.2 Synthesis of Ionophore

1,8-Anthracenemethanamine was synthesized as follows: To a stirred solution of 1,8-bis(aminomethyl)anthracene (0.1 g, 0.42 mmol) in 20 mL of methylene chloride, a solution of 2,3,4,6-tetra-0-acetyl-α-D-glycopyranosyl-isothiocynate (0.33 g, 0.85 mmol) was added dropwise [32]. The mixture was stirred for 1 h under N$_2$ atmosphere at room temperature. After completion of reaction, the solvent was removed under vacuum afforded the crude product and the residue was purified by chromatography on silica gel eluted with ethyl acetate/hexane/methanol (1:1:0.1) to yield 0.3 g (70 %) of anthracene thiourea derivative as a yellow solid (Figure 1).

$^3$H NMR (acetone-$d_6$, ppm) δ 1.84 (s, 6H), 1.94 (s, 6H), 1.96 (s, 6H), 1.99 (s, 6H), 3.99–4.07 (m, 4H), 4.26 (dd, $J=4.5$ Hz, 2H), 4.93–5.04 (m, 4H), 5.39 (t, $J=9.6$ Hz, 2H), 5.49 (d, 4H), 6.00 (t, $J=6.9$ Hz, 2H), 7.40 (d, $J=8.4$ Hz, 2H), 7.47 (t, $J=7.7$ Hz, 2H), 7.57 (d, $J=6.9$ Hz, 2H), 7.93 (t, 2H), 8.03 (d, $J=8.1$ Hz, 2H), 8.61 (s, 1H), 8.92 (s, 1H);

$^{13}$C NMR (CD$_3$CN, ppm) δ 20.10, 20.17, 20.24, 46.63, 62.13, 68.57, 71.08, 73.47, 78.52, 82.34, 118.35, 125.44, 125.76, 128.20, 128.41, 129.82, 131.97, 134.40, 170.18, 170.38, 170.61, 171.00, 184.42; IR (KBr) cm$^{-1}$; 3360.35, 2925.34, 1547.59, 1377.89, 1234.22, 600.72; HR-ESI-MS $m/z$ = 1015.2987 (M+H)$^+$, calcd for C$_{46}$H$_{55}$N$_4$O$_{18}$S$_2$: 1015.2946.
2.3 Sensor Preparation
Poly(vinyl chloride) was used as a mechanical support for the preparation of the sensor membranes containing ionophore, anion exchanger as well as different solvent mediators. The obtained electroanalytical data were compared to select the better membrane composition. Varying amounts of the ionophore, PVC, TDMACl, and solvent mediators were mixed in 1 mL of THF. The resulting mixture was taken in a closed container and shaken vigorously for about 8 h in a shaker to get a homogeneous solution. The polymer membrane was prepared by dropping 10 μL of the membrane solution cocktail on a thoroughly cleaned and polished graphite electrode and evaporating the solvent for 24 h in a closed cabinet at room temperature. The sensor was finally conditioned for one day by soaking it in a 1.0×10^{-2} M K_2HPO_4 solution (pH 7.4).

Conventional ISEs were also prepared by using the standard procedure [33] and compared for their electrochemical behavior with the developed all solid state sensor.

2.4 EMF Measurements
The electrochemical cell used for the potential measurement was composed as follows: Ag/AgCl/saturated KCl/sample solution/solid membrane on a carbon rod. Despite the utmost care was taken during the preparation of membranes, the thickness and the internal resistance of the resulting membranes varied in each case. Since the standard potentials between electrodes were different, the normalization of the potentials was necessary to allow comparisons of the sensor responses. The potential for each test electrode in 1.0×10^{-2} M K_2HPO_4 solution was set to 0 mV. Standard monohydrogen phosphate solutions were prepared by serial dilution of a 0.1 M K_2HPO_4 solution and then titrated with KOH to pH 7.4. The pHs of solutions under investigation were continuously monitored and maintained throughout. The phosphate activity was derived from the Debye–Hückel limiting law. These were calculated using the total phosphate concentration, the pH of standard solution, the equilibrium constant of different phosphate species and the ionic strength. The selectivity coefficients of electrode for the monohydrogen phosphate ion with respect to other anions were determined by using the separate solution method using 10^{-3} M concentration of interfering ions. All the solutions used for the determination of selectivity coefficient values were adjusted to pH 7.40±0.02. The detection limit for monohydrogen phosphate was determined by the IUPAC recommended method [34].

3 Results and Discussion
3.1 Interaction Between Ionophore and Monohydrogenphosphate Ions
Ion sensors for hydrophilic ions, phosphate or sulfate ions are usually based on ion exchangers [35]. In order to prepare a sensor for these ions, the membrane should contain ionophore molecules that must be capable to bind these ions specifically. Neutral anion ionophores containing hydrogen bond forming moieties or immobilized Lewis acidic binding sites can be applied for this purpose.

It is especially challenging to achieve the useful selectivity for a strongly hydrophilic anion, particularly in the phosphate species, since their unfavorable standard free enthalpies transfer from samples to ISE membranes have been overcompensated by selective complexation [36]. The interaction of monohydrogen phosphate ion with the ionophore might be due to the H-bond formation between sec-amine and oxygen of phosphate ion (Figure 2). This is further confirmed as the sensor exhibited selective behavior towards monohydrogen phosphate ions.

3.2 Characterization of the Electrode Response
The present ionophore based-sensor shows a linear potentiometric response towards phosphate ions with an average slope of 30.8±1.0 mV/decade of activity (Figure 3). This is important to mention that the conventional ISEs (containing internal reference solution) prepared with the same membrane composition produced the similar calibration curve. The pH values of test and standard solutions were adjusted to 7.4 in all the experi-

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Fig. 2. Interaction mechanism of anion-receptor

Anthracene thiourea derivative

$\text{HPO}_4^{2-} : -1.01 \text{ kcal/mol}$

$\text{HPO}_4^{2-} : -78.3518 \text{ kcal/mol}$

Anthracene thiourea derivative

$\text{26.2703} \text{ kcal/mol}$
ments. Although the value of slope itself suggests that the sensor may be responding towards divalent monohydrogen phosphate ions with a near Nernstian slope, however, only the favorable slope value is not sufficient for the confirmation of the response behavior of the sensor because it may be another possibility that the sensor is showing a poor response towards $H_2PO_4^-/CO_3^{2-}$ ions with a sub-Nernstian slope. The distribution of phosphate species is pH dependent; therefore, the relative amount of one phosphate form in a solution increases and the amount of the other form decreases as the pH of the solution varies. A method of varying the pH while keeping the total concentration of anion being measured constant was adopted to determine which of the phosphate species is actually being responded by the sensor [18]. It was carried out by monitoring the response of the sensor in a solution containing 0.05 mM total phosphate ion concentration at varied pHs. At the lower pH, the monovalent dihydrogen phosphate form is dominant, but as the pH approaches neutral, the $H_2PO_4^-/CO_3^{2-}$ form changes into $HPO_4^{2-}/CO_3^{2-}$ and the divalent monohydrogen form becomes dominant. By considering the near Nernstian slope of the sensor and its response towards the pH variation, it is concluded that the sensor is responding towards dibasic phosphate ions.

### 3.3 Optimization of the Electrode Composition

The response of the proposed sensor was observed for its selective behavior towards different types of anions i.e. $HPO_4^{2-}$, $SO_4^{2-}$, $OAc^-$, $ClO_4^-$, $CO_3^{2-}$, and $Cl^-$. The best response was recorded for $HPO_4^{2-}$ ions. The sensor exhibited the widest working concentration range as well as near Nernstian slope for monohydrogen phosphate anions (Figure 3). It is well established that the nature and amount of ionophore as well as of the plasticizers, as well as an anion exchanger, significantly affect the sensitivity, and the selectivity of the ion-selective electrode. The effect of the addition of these membrane ingredients was investigated by preparing a number of electrode membranes having different compositions. Optimization of the membrane ingredients was done to develop the best performing electrodes and the results obtained for them are presented in Table 1.

The presented data shows that the membranes containing 8.0 wt% of the ionophore (sensor no. 3) showed the

![Fig. 3. Potentiometric response of the ionophore containing ASISEs towards different anions.](image)

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Composition</th>
<th>Working concentration range (M)</th>
<th>Slope (mV/dec of activity)</th>
<th>Response time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 NPOE, 63</td>
<td>$6.0 \times 10^{-6} - 1.0 \times 10^{-3}$</td>
<td>29.6</td>
<td>120</td>
</tr>
<tr>
<td>2</td>
<td>6 NPOE, 60</td>
<td>$4.0 \times 10^{-7} - 1.0 \times 10^{-3}$</td>
<td>30.0</td>
<td>128-30</td>
</tr>
<tr>
<td>3</td>
<td>8 NPOE, 58</td>
<td>$1.0 \times 10^{-7} - 1.0 \times 10^{-3}$</td>
<td>30.8</td>
<td>12-80</td>
</tr>
<tr>
<td>4</td>
<td>9 NPOE, 57</td>
<td>$1.0 \times 10^{-7} - 1.0 \times 10^{-3}$</td>
<td>30.8</td>
<td>12-80</td>
</tr>
<tr>
<td>5</td>
<td>8 DOA, 58</td>
<td>$8.0 \times 10^{-9} - 1.0 \times 10^{-3}$</td>
<td>26.0</td>
<td>60-80</td>
</tr>
<tr>
<td>6</td>
<td>8 BBPA, 58</td>
<td>$5.0 \times 10^{-5} - 1.0 \times 10^{-3}$</td>
<td>23.7</td>
<td>60-80</td>
</tr>
</tbody>
</table>

Table 1. Compositions of the response characteristics of the fabricated phosphate sensor. All determinations were carried out 3 times.
near-Nernstian slope (30.8 ± 1.0 mV/decade of activity) within the concentration range of 1.0×10^{-7}–1.0×10^{-3} M. If the weight percent of ionophore is less than 8, the working concentration range of the sensor becomes narrower i.e. 4.0×10^{-7}–1.0×10^{-3} M. This was obviously due to the decreased number of available ionophore molecules within the membrane. If the weight percent of the ionophore is taken more than 8, there is no change observed in any of the response characteristics of the sensor. Out of the used plasticizers, the most polar one, NPOE, was found the most effective in comparison with DOA (sensor no. 5) and BBPA (sensor no. 6). This indicates the effect of plasticizer on the dielectric constant of the membrane as well as on the mobility of the ionophore and its complex. The membranes prepared with DOA, and BBPA plasticizers exhibited relatively narrow working concentration range of 8.0×10^{-6}–1.0×10^{-3} M, and 5.0×10^{-5}–1.0×10^{-3} M, respectively. Out of the all prepared sensors, sensor no. 3, having a membrane composition of 8:32.58:2 (Ionophore:PVC:NPOE:TDMACI) wt%, shown the widest working concentration range of 1.0×10^{-7}–1.0×10^{-3} M with a near-Nernstian slope of 30.8 ± 1.0 mV/decade of activity.

### 3.4 Response Time and Stability

The time needed for getting the 95% of the full potential change after successive addition of a series of metal ion solutions is called as response time of that sensor. Sensor no. 3 exhibited the quickest response with the response time of 8–10 s (Table 1). The response behavior of the membrane electrodes remained unchanged when the potentials were recorded either from higher to lower concentration or vice versa.

The life span of ASISE was studied by monitoring the change in the electrode slope and the linear response range according to time. The electrode did not show significant changes in the slope or in the working concentration range for over twelve weeks which is quite satisfactory. This is worth mentioning that the life time of the conventional electrode with the same composition of the membrane was found lesser than ASISE. It could work only for 8–9 weeks. This may be due to the fact that in conventional ISEs, both sides of the membrane are in contact with the aqueous solution while the only one side of the membrane in ASISE is exposed to the solution. This one side exposure of the membrane of the ASISE towards the aqueous solution slows down the process of slow but continuous leaching of ionophore from the membrane into the solution. The electrodes were stored in a 10^{-2} M HPO_{4}^{2-} solution (pH 7.4) between experiments. The standard deviation of potentials for 10 measurements at a fixed concentration of 1.0×10^{-4} M was found to be ±1.0 mV. Of all the prepared sensors, the sensor no. 3 remained the best performing electrode as it exhibited the widest working concentration range, lowest response time, and the stable potential response. Therefore, the same was chosen for further experiments.

### 3.5 Selectivity

When analyzing real samples with ion sensors, the response of the electrode to the primary ion can be affected by the presence of other ions of the similar charge. The influence of interfering ions on the response behavior of sensors is usually described in terms of log values of selectivity coefficients i.e. $K_{\text{AI}}$. The selectivity coefficients were obtained by the separate solution method [33] using 10^{-3} M concentration of the expected interfering anions (Table 2).

The observed selectivity coefficients for OAc$^{-}$, NO$_{3}^{-}$, Cl$^{-}$, I$^{-}$, Br$^{-}$, and SO$_{4}^{2-}$ are significantly lower, which indicate the high selectivity of the electrode for monohydrogen phosphate ions over these anions. The selectivity sequence for this electrode was observed as: HPO$_{4}^{2-} >$ SO$_{4}^{2-} >$ Cl$^{-} >$ NO$_{3}^{-} >$ I$^{-} >$ Br$^{-} >$ OAc$^{-} >$ NO$_{3}^{-} >$ Cl$^{-}$, which is not consistent with the Hofmeister series. This anti-Hofmeister selectivity response pattern clearly reveals the mechanism of recognition of HPO$_{4}^{2-}$ which takes place via hydrogen bonds of the ionophore (Figure 2). The selectivity coefficients obtained for the proposed sensor are compared with earlier reported phosphate ion selective electrodes (Table 3).

It shows that the proposed phosphate ion selective electrode displays comparable [20,23,26] and even better selectivity than some of the previously reported phosphate ion selective electrodes [12,15,21].

### 3.6 Analytical Applications

Standard reference material, SRM 1548 which was a complex mixture of different foods which represented the daily intake, was selected for the real sample analysis. 1.0 g of homogenized sample was weighed into acid cleaned and thoroughly washed and thoroughly dried quartz tube and placed into a muffle furnace. The temperature was raised slowly to 480°C and the sample was kept overnight [37]. The obtained ash was treated with 1 mL concentrated nitric acid and then dried thoroughly on a heating block. It was placed again into the muffle furnace at 480°C overnight. The sample ash was diluted to a final volume of 25 mL by adding deionized water. The pH of the obtained solution was adjusted to 7.4 and the potential response was recorded. The results exhibited that the phosphorous concentration present in the test
OAC phosphate solution was titrated against a 1.0 M barium chloride solution. An indicator electrode for the determination of barium ion phosphate, suggesting that the electrode can be used as an in-point corresponding to the stoichiometry of barium phosphate in the standard reference material. Apart from being an advantageous all solid state electrode, it exhibited better or comparable response time, working concentration range, and life time than many of the existing electrodes.

Table 3. Comparison of potentiometric selectivity coefficients, obtained for the sensor no. 3 with the existing monohydrogen phosphate selective electrodes with References [12–27]. NM: not mentioned.

<table>
<thead>
<tr>
<th>Interfering ion</th>
<th>This work</th>
<th>[12]</th>
<th>[14]</th>
<th>[15]</th>
<th>[20]</th>
<th>[21]</th>
<th>[23]</th>
<th>[26]</th>
<th>[27]</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO₄²⁻</td>
<td>−1.3</td>
<td>NM</td>
<td>−4.3</td>
<td>−2.3</td>
<td>−1.5</td>
<td>NM</td>
<td>−1.8</td>
<td>−1.8</td>
<td>−2.5</td>
</tr>
<tr>
<td>ClO₄⁻</td>
<td>−2.7</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
<td>13</td>
<td>−2.3</td>
<td>−3.8</td>
</tr>
<tr>
<td>I⁻</td>
<td>−2.4</td>
<td>−0.6</td>
<td>−0.04</td>
<td>−1.3</td>
<td>−2.2</td>
<td>−0.3</td>
<td>4.0</td>
<td>−2.3</td>
<td>−3.3</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>−2.0</td>
<td>−1.7</td>
<td>−2.4</td>
<td>−1.3</td>
<td>−2.2</td>
<td>−0.3</td>
<td>4.0</td>
<td>−2.3</td>
<td>−3.3</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>−1.8</td>
<td>−2.5</td>
<td>−3.0</td>
<td>−1.8</td>
<td>−2.9</td>
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</tr>
<tr>
<td>OAC⁻</td>
<td>−2.4</td>
<td>−2.3</td>
<td>−3.2</td>
<td>NM</td>
<td>−1.7</td>
<td>NM</td>
<td>−1.4</td>
<td>−1.5</td>
<td>−3.2</td>
</tr>
</tbody>
</table>

4 Conclusions

An all solid state monohydrogen phosphate ion sensor has been developed by using anthracene thiourea derivative, a macrocyclic compound, as an ionophore in poly(vinyl chloride) (PVC) membrane matrix. It exhibited a near Nernstian response in a wider linear range. It is free from the interference due to the presence of the common bivalent and monovalent ions and shows highly selective response towards HPO₄²⁻ ions. It could be successfully used as an indicator electrode for the end point determination in the potentiometric titration of phosphate solution against the standard barium chloride solution as well as to determine the concentration of phosphate in the standard reference material. Apart from being an advantageous all solid state electrode, it exhibited better or comparable response time, working concentration range, and life time than many of the existing electrodes.

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